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Biotimer assay: A reliable and rapid method for the evaluation of central venous catheter microbial colonization

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ABSTRACT

Adherent bacteria and biofilm frequently colonize central venous catheters (CVCs). CVC colonization is correlated to infections and particularly to bloodstream ones. The classical microbiological methods to determine of CVC colonization are not fully reliable and are time-consuming. BioTimer Assay (BTA) is a biological method already used to count bacteria adherent to abiotic surfaces and biofilm without sample manipulation. BTA employs specific reagents whose color changed according to bacterial metabolism. BTA is based on the principle that a metabolic reaction will be faster when more bacteria are present in the sample. Therefore, the time required for color changes of BTA reagents determines the number of bacteria present in the sample through a correlation line. Here, for the first time, we applied BTA and a specifically developed laboratory procedure to evaluate CVC colonization in comparison with the routine microbiological method (RMM). 125 CVCs removed from patients for suspected catheter-related bloodstream infection (CRBSI) or at hospital discharge were examined. BTA was reliable in assessing sterility and CVC colonization (100% agreement with RMM) and in recognizing the presence of fermenting or non-fermenting bacteria (97.1% agreement with RMM) shortening the analytical time by between 2- and 3-fold. Moreover, the reliability of BTA as early alert of CRBSI was evaluated. The sensitivity, specificity, positive, and negative predictive values for BTA as an early alert of CRBSI were 100, 40.0, 88.8 and 100%, respectively.

In conclusion, BTA and the related laboratory procedure should be incorporated into routine microbiological methods since it can be considered a reliable tool to evaluate CVC colonization in a very short time and a rapid alert for CRBSIs.

1. Introduction

Central venous catheters (CVCs) are a mainstay for management of critically ill patients. However, CVCs may be colonized on intra- and extra-luminal surfaces by microorganisms in adherent and biofilm lifestyle (Dobbins et al., 2003; Gominet et al., 2017). CVC colonization is correlated to infectious episodes and, in particular, the catheter-related bloodstream infections (CRBSIs) are characterized by high rates of morbidity and mortality (Pratt et al., 2001; Chopra et al., 2013; Gahlot et al., 2014; Yousif et al., 2015). Moreover, the biofilms colonizing CVCs show antimicrobial resistance significantly higher than the planktonic counterparts (Donlan and Costerton, 2002; Hall-Stoodley et al., 2004; Pantanella et al., 2008) prolonging the length of hospitalization and increasing healthcare costs (Kaye et al., 2014; Gahlot et al., 2014).

The microbiological methods for the routine analysis of CVC colonization are semi-quantitative and actually based on the roll-plate technique (Maki et al., 1977; Mermel et al., 2009) or on the detachment of adherent microbes by vortex or sonication and the count of the detached microorganisms by the colony forming unit (CFU) technique (Cleri et al., 1980; Sherertz et al., 1990; Wengrovitz et al., 1991). Unfortunately, no one of these methods is fully reliable. Indeed, the roll-plate technique does not determine the intra-luminal colonization of CVCs (Dobbins et al., 2003; Mermel et al., 2009; Guembe et al., 2016; Gominet et al., 2017) and the vortex or sonication methods do not ensure the detachment of all microbes (Pantanella et al., 2013; Freitas

Abbreviations: BTA, BioTimer Assay; CVCs, central venous catheters; RMM, routine microbiological method; CRBSI, catheter-related bloodstream infection

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[1] This manuscript is dedicated to Maria Cristina Ghezzi who prematurely passed away.

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et al., 2014). It is evident that a rapid and reliable microbiological method for the early determination of CVC colonization is extremely important. BioTimer Assay (BTA) is a biological method that counts adherent bacteria and biofilm without sample manipulation as vortex or sonication to detach biomass and cultivation, thus overcoming the above-mentioned limits (Berlutti et al., 2003; Pantanella et al., 2008; Pantanella et al., 2011; Berlutti et al., 2014; Srivastava and Bhargava, 2016). In particular, BTA is based on the principle that a metabolic reaction will be faster when more bacteria are present in the sample. BTA employs specific reagents whose color changes according to bacterial metabolism. The time required for color changes of BTA reagents is inversely related to initial bacterial concentration. Therefore, the time for color change determines the number of bacteria present in the sample at Time 0 through genus-specific correlation lines. In addition, BTA was successfully employed to evaluate biofilm colonization of medical devices (Hess et al., 2011; Wells et al., 2011; Romeo et al., 2015).

Here, for the first time, BTA was applied to evaluate microbial colonization of CVCs removed from patients hospitalized at the University Hospital Policlinico Umberto I, Rome, Italy. For this purpose, a specific laboratory procedure was developed. The performance of BTA and the related laboratory procedure was compared with the method of Cleri et al. (1980) routinely employed at the Clinical Microbiological Laboratory of the same hospital. Furthermore, the correlation between BTA results and CVC-related bloodstream infection (CRBSI) diagnosis was investigated. The data presented strongly indicate that BTA can be usefully employed to evaluate CVC colonization and can represent an alert tool for CRBSI.

2. Materials and methods

2.1. Bacterial strains and culture media

Staphylococcus epidermidis ATCC 12228 and *Pseudomonas aeruginosa* ATCC 15692 (PAO1) were streaked on 5% Columbia blood agar (CBA) plates (Oxoid LTD, England) before the experiments to check purity.

2.2. BioTimer Assay (BTA)

BioTimer Assay (BTA) allows counting bacteria in planktonic, adherent and biofilm lifestyle (Berlutti et al., 2003; Pantanella et al., 2008; Pantanella et al., 2011; De Giusti et al., 2011; Berlutti et al., 2014). BTA employs two specific reagents: BioTimer-phenol red (BT-PR) and BioTimer-resazurin (BT-RZ) whose color changes due to microbial metabolism (Berlutti et al., 2003; Pantanella et al., 2008; Pantanella et al., 2011). In particular, BT-PR reagent changed red-to-yellow due to reagent acidification when inoculated with fermenting bacteria and BT-RZ reagent changed blue-to-pink due to redox reaction when inoculated with fermenting or non-fermenting bacteria (Pantanella et al., 2011). BT-PR and BT-RZ reagents were prepared as previously described with slight modification (Pantanella et al., 2008; Pantanella et al., 2011; Berlutti et al., 2014). Briefly, to prepare BR-PR reagent, 3.7 g of Brain Heart Infusion (BHI; Oxoid Ltd., UK) were dissolved in 940 ml of distilled water. After sterilization at 115 °C for 15 min, 50 ml of 10% filtered glucose solution and 10 ml of filtered 0.25% phenol red (Sigma Aldrich, Italy) were added. If necessary, the pH was adjusted to 7.2 ± 0.1. The final BT-PR reagent appeared clear and red. To prepare the BT-RZ reagent, 10 ml of freshly prepared and filtered 0.1% aqueous resazurin solution (Sigma-Aldrich, Italy) was added to 990 ml sterile BHI broth prepared as above described (Oxoid). If necessary, the pH was adjusted to pH 7.0 ± 0.1. The final BT-RZ reagent appeared clear and blue.

The time required for color change of BTA reagents is correlated to initial bacterial concentration by correlation lines. To draw the correlation lines, serial two-fold dilutions of planktonic overnight broth cultures of *S. epidermidis* and of *P. aeruginosa* in 1 ml of BT-PR and BT-RZ reagents, respectively, were performed in 24-well plates (BD, Italy), and simultaneously counted using the colony forming unit (CFU) method. Briefly, to carry out the CFU method, bacterial suspensions were diluted in sterile saline solution and plated on BHI plates. After 24 h of incubation, colonies were counted. The number of CFUs in the initial bacterial suspensions was determined by multiplying the number of counted CFUs on BHI plates with the dilution factor. The time (in hours) required for color changing of the inoculated BT-PR and BT-RZ reagents was recorded and plotted versus the corresponding CFU values (Fig. 1). The equations and the linear correlation coefficients describing the correlation lines were calculated for each microorganism on the whole data set and were: $y = -0.5045x + 7.6959$ $R^2 = 0.9891$ for *S. epidermidis* and $y = -0.4675x + 8.5421$ and $r^2 = 0.9968$ for *P. aeruginosa*. As the correlation lines correlated the time for the color change of BTA reagents with the number of planktonic CFUs, the number of bacteria in biofilm was expressed as planktonic-equivalent CFUs (PE-CFUs) (Pantanella et al., 2008).

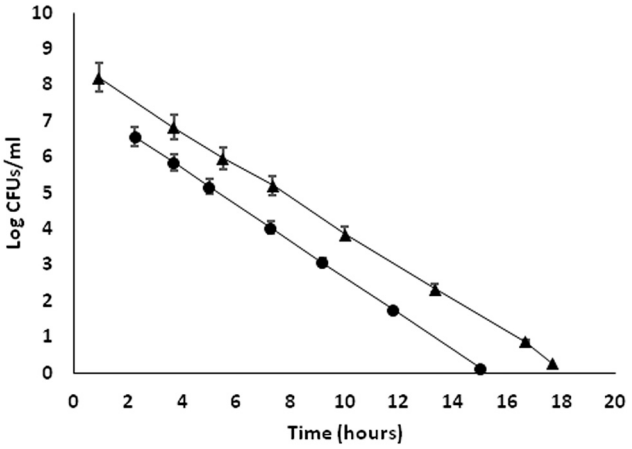

Fig. 1. BioTimer Assay correlation lines.
Legend: Correlation lines of *S. epidermidis* ATCC 12228 (circles) and of *P. aeruginosa* ATCC 15692 (triangles) in BT-PR and BT-RZ reagents, respectively.

2.3. Experimental colonization of central venous catheters

Sterile CVCs (Multi-Lumen Central Venous Catheterization Set, Arrow Italy) were aseptically cut in 1 cm-segments and immersed in sterile saline (0.9% NaCl) supplemented with 2% human serum pooled from healthy volunteers. After 30 min incubation, the CVC pieces were washed three times with sterile saline and transferred into sterile 24-well flat-bottom plates. A total of 1 ml of BHI broth containing about 10^4 CFUs of planktonic log phase cultures of *S. epidermidis* ATCC 12228 or *P. aeruginosa* ATCC 15692 (PAO1) were added to each well. CVCs were incubated for 2 and 24 h to have adherent bacteria and biofilm, respectively. After incubation, bacteria were counted using the culture method according to Cleri et al. (1980) with slight modification. Briefly, the CVC segments were transferred into sterile tubes containing 1 ml of BHI broth and then vortexed for 30 s to detach bacteria. Detached bacteria were counted by CFU method on BHI agar. To evaluate the residual adherent bacteria after vortex, the CVC segments were immersed in 1 ml of BT-RP and BT-RZ reagents and counted by BTA.

2.4. Central venous catheters collection

CVCs were randomly selected among those received at the Clinical Microbiology laboratory at “Azienda Policlinico Umberto I”, Hospital of Sapienza University of Rome, Italy during a two years period (March 2014 to March 2016). CVCs were removed from hospitalized patients either at the end of the clinical therapy or for the clinical suspicion of CRBSI (Mermel et al., 2009).